

CLAIMS

What is claimed is:

1. A method using probes and/or amplification primers which are specific,
5 ubiquitous and sensitive for determining the presence and/or amount of nucleic acids:
- from a bacterial antibiotic resistance gene selected from the group consisting
of *bla_{tem}*, *bla_{shv}*, *bla_{rob}*, *bla_{oxB}*, *bla_Z*, *aadB*, *aacC1*, *aacC2*, *aacC3*, *aac6'-IIa*, *aacA4*,
aad(6'), *vanA*, *vanB*, *vanC*, *msrA*, *satA*, *aac(6')-aph(2'')*, *vat*, *vga*, *ermA*, *ermB*, *ermC*,
mecA, *int* and *sul*, and
10 - from specific bacterial and fungal species selected from the group consisting
of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*,
Streptococcus agalactiae, *Candida albicans*, *Enterococcus* genus, *Neisseria* genus,
Staphylococcus genus, *Streptococcus* genus and *Candida* genus,
in any sample suspected of containing said bacterial and/or fungal nucleic acids,
15 wherein each of said nucleic acid or variant or part thereof comprises a selected
target region hybridizable with said probes or primers;
said method comprising the following steps: contacting said sample with said
probes or primers under uniform conditions of hybridization or amplification, and
detecting the presence and/or amount of hybridized probes or amplified products as
20 an indication of the presence and/or amount of said specific bacterial and/or fungal
species or genus simultaneously with said bacterial antibiotic resistance genes.
2. A method according to claim 1, which further makes use of probes and/or
primers which are specific, ubiquitous and sensitive for simultaneously determining the
25 presence and/or amount of nucleic acids from any bacterium or fungus.
3. The method of claim 1, which is performed directly from a test sample.
4. The method of claim 1, which is performed directly from a test sample
30 consisting of a bacterial and/or fungal culture or suspension.
5. The method of claim 1, wherein said nucleic acids are amplified by a method
selected from the group consisting of:
35 a) polymerase chain reaction (PCR),
b) ligase chain reaction (LCR),
c) nucleic acid sequence-based amplification (NASBA),
d) self-sustained sequence replication (3SR),
e) strand displacement amplification (SDA),
f) branched DNA signal amplification (bDNA),

- g) transcription-mediated amplification (TMA),
- h) cycling probe technology (CPT),
- i) nested PCR, and
- j) multiplex PCR.

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6. The method of claim 6, wherein said nucleic acids are amplified by PCR.

7. The method of claim 6, wherein the PCR protocol achieves within one hour under uniform amplification conditions the determination of the presence of said nucleic acids by performing for each amplification cycle an annealing step of thirty seconds at 45-55°C and a denaturation step of only one second at 95°C without any time specifically allowed to an elongation step.

8. A method for the detection, identification and/or quantification of a microorganism selected from the group consisting of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species, *Streptococcus* species and *Candida* species, directly from a test sample or from bacterial and/or fungal cultures, which comprises the following steps:

a) depositing and fixing on an inert support or leaving in solution the said microorganism DNA of the sample or of a substantially homogeneous population of said microorganism isolated from this sample, or

inoculating said sample or said substantially homogeneous population of microorganism isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or said isolated microorganism to release the said microorganism DNA,

said microorganism DNA being made in a substantially single-stranded form;

b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleic acid which nucleotide sequence is selected from the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 120, 131 to 134, 31, 140 to 143, 32 to 36, 120 to 124, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species, *Streptococcus* species and *Candida* species, respectively, under conditions such that the nucleic acid of said probe can selectively hybridize with said microorganism DNA, whereby a hybridization complex is formed; and

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c) detecting the presence of said hybridization complex on said inert support or in said solution as an indication of the presence and/or amount of said microorganism, in said test sample.

9. A method for detecting the presence and/or amount of a microorganism selected from the group consisting of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species, *Streptococcus* species and *Candida* species, in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said microorganism DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 120, 131 to 134, 31, 140 to 143, 32 to 36, 120 to 124, respectively with regard to said microorganism, a sequence complementary thereof, and a variant thereof;

b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of said microorganisms, in said test sample.

10. The method of claim 9 wherein said pair of primers is defined in SEQ ID NOs: 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, 13 and 14, 15 and 16, 17 to 20, 21 and 22, respectively, for each of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species and *Streptococcus* species.

11. A method for detecting the presence and/or amount of any bacterium directly from a test sample or a bacterial culture, which comprises the following steps:

a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogeneous population of bacteria isolated from this sample, or

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a) treating said sample with an aqueous solution containing a pair of primers having a sequence selected within the nucleotide sequences defined in SEQ ID NOs:

107 and 108, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, and a variant thereof, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial *tuf* gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;

b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence; and
d) determining the nucleotide sequence of the said amplified target sequence by using any DNA sequencing method.

15. A method for detecting the presence and/or amount of any fungus directly from a test sample or a fungal culture, which comprises the following steps:

a) depositing and fixing on an inert support or leaving in solution the fungal DNA of the sample or of a substantially homogeneous population of fungi isolated from this sample, or

inoculating said sample or said substantially homogeneous population of fungi isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or isolated fungi to release the fungal DNA,

said fungal DNA being made in a substantially single-stranded form;

b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleotide sequence selected from the group consisting of SEQ ID NOs: 120 to 124, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of any fungus, under conditions such that the nucleic acid of said probe can selectively hybridize with said fungal DNA, whereby a hybridization complex is formed; and

c) detecting the presence of said hybridization complex on said inert support or in said solution as an indication of the presence and/or amount of any fungus in said test sample.

16. A method for detecting the presence and/or amount of any fungus in a test sample which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of any fungal DNA that contains a target sequence, and the other of said primers being

capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 120 to 124, a sequence complementary thereof, and a variant thereof;

b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of any fungus in said test sample.

17. A method for obtaining *tuf* sequences from any fungus directly from a test sample or a fungal culture, which comprises the following steps:

a) treating said sample with an aqueous solution containing a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 109 and 172, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, and a variant thereof, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said fungal *tuf* gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;

b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence; and

d) determining the nucleotide sequence of the said amplified target sequence by using any DNA sequencing method.

18. A method as defined in any one of claims 1 to 8, which comprises the evaluation of the presence of a bacterial resistance mediated by a bacterial antibiotic resistance gene selected from the group consisting of *bla_{oxa}*, *blaZ*, *aac6'-IIa*, *ermA*, *ermB*, *ermC*, *vanB*, *vanC*, directly from a test sample or a bacterial culture, which comprises the following steps:

a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogeneous population of bacteria isolated from this sample, or

inoculating said sample or said substantially homogeneous population of bacteria isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or isolated bacteria to release the bacterial DNA,

said bacterial DNA being made in a substantially single-stranded form;

b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleotide sequence having at least 12 nucleotide in length is selected from the group consisting of SEQ ID NOs: 110, 111, 112, 113, 114 115, 116, 117, a sequence complementary thereof, and a variant thereof, which specifically hybridizes with said bacterial antibiotic resistance gene, respectively; and

c) detecting the presence of a hybridization complex as an indication of a bacterial resistance mediated by said one of said bacterial antibiotic resistance genes.

19. A method as defined in any one of claims 1 to 7, 9 and 10, which comprises the evaluation of the presence of a bacterial resistance mediated by a bacterial antibiotic resistance gene selected from the group consisting of *bla_{oxa}*, *blaZ*, *aac6'-IIa*, *ermA*, *ermB*, *ermC*, *vanB*, *vanC*, directly from a test sample or a bacterial culture, which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 110, 111, 112, 113, 114, 115, 116, 117, respectively with regard to said bacterial antibiotic resistance gene, a sequence complementary thereof, and a variant thereof;

b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance mediated by one of said bacterial antibiotic resistance genes.

20. A method as defined in any one of claims 1 to 7, 9 and 10, which comprises the evaluation of the presence of a bacterial resistance gene selected from the group consisting of *bla_{tem}*, *bla_{shv}*, *bla_{rob}*, *bla_{oxa}*, *blaZ*, *aadB*, *aacC1*, *aacC2*, *aacC3*, *aac6'-IIa*, *aacA4*, *aad(6')*, *vanA*, *vanB*, *vanC*, *msrA*, *satA*, *aac(6')-aph(2'')*, *vat*, *vga*, *ermA*, *ermB*, *ermC*, *mecA*, *int* and *sul*, directly from a test sample or a bacterial culture, which comprises the following steps:

a) treating said sample with an aqueous solution containing at least one pair of primers having a sequence selected in the group consisting of SEQ ID NOs: 37 to 40, 41 to 44, 45 to 48, 49 and 50, 51 and 52, 53 and 54, 55 and 56, 57 and 58, 59 to 60,

61 to 64, 65 and 66, 173 and 174, 67 to 70, 71 to 74, 75 and 76, 77 to 80, 81 and 82, 83 to 86, 87 and 88, 89 and 90, 91 and 92, 93 and 94, 95 and 96, 97 and 98, 99 to 102, 103 to 106, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, a variant thereof, and mixtures thereof, one of said primers of said pair being capable of hybridizing selectively with one of the two complementary strands of its respective bacterial antibiotic resistance gene that contains a target sequence, and the other of said primers of said pairs being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;

b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and

c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance mediated by one of said bacterial antibiotic resistance genes.

21. A nucleic acid having the nucleotide sequence of any one of SEQ ID NOs: 26 to 36, 110 to 117, a part thereof, a sequence complementary thereof, and variant thereof which, when in single-stranded form, ubiquitously and specifically hybridizes with a target bacterial or fungal DNA as a probe or as a primer.

22. An oligonucleotide having the nucleotide sequence of any one of SEQ ID NOs: 1 to 25, 37 to 71, 73 to 109 and 172 to 174, a part thereof, a sequence complementary thereof, and variant thereof, which ubiquitously and specifically hybridizes with a target bacterial or fungal DNA as a probe or as a primer.

23. A recombinant plasmid comprising a nucleic acid as defined in claim 22.

24. A recombinant host which has been transformed by a recombinant plasmid according to claim 23.

25. A recombinant host according to claim 24 wherein said host is *Escherichia coli*.

26. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the microbial species and/or genera selected from the group consisting of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species, *Streptococcus* species and *Candida* species, comprising any suitable combination of probes of at

least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 26 to 36, 120 to 124, 131 to 134, 140 to 143, sequences complementary thereof, and variants thereof.

27. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the microbial species and/or genera selected from the group consisting of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species, *Streptococcus* species and *Candida* species, comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 26 to 36, 120 to 124, 131 to 134, 140 to 143, sequences complementary thereof, and variants thereof.

28. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the microbial species and/or genera selected from the group consisting of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species and *Streptococcus* species, comprising any suitable combination of primers selected from the group consisting of SEQ ID NOs: 1 to 22, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof.

29. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of *bla_{oxa}*, *blaZ*, *aac6'-IIa*, *ermA*, *ermB*, *ermC*, *vanC*, comprising any suitable combination of probes of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 110 to 115 and 117, sequences complementary thereof, and variants thereof.

30. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of *bla_{oxa}*, *blaZ*, *aac6'-IIa*, *ermA*, *ermB*, *ermC*, *vanC*, comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 110 to 115 and 117, sequences complementary thereof, and variants thereof.

31. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium and/or fungus, comprising any combination of probes of at least 12

nucleotides in length selected upon alignment of conserved nucleotides of at least two sequences of the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof.

- 5 32. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium and/or fungus, comprising any suitable combination of primers of at least 12 nucleotides in length selected upon alignment of conserved nucleotides of at least two sequences the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof.

10 33. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium, comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof.

15 34. A diagnostic kit, as defined in claim 26, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.

20 35. A diagnostic kit, as defined in claim 27, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.

25 36. A diagnostic kit, as defined in claim 28, further comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.

30 37. A diagnostic kit, as defined in claim 26, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of *bla_{oxa}*, *bla_Z*, *aac6'-IIa*, *ermA*, *ermB*, *ermC*, *vanB*, *vanC*.

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38. A diagnostic kit, as defined in claim 27, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of *bla_{oxa}*, *blaZ*, *aac6'-IIa*, *ermA*, *ermB*, *ermC*, *vanB*, *vanC*.

39. A diagnostic kit, as defined in claim 28, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 37 to 106, 173 and 174, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of *bla_{tem}*, *bla_{rob}*, *bla_{shv}*, *bla_{oxa}*, *blaZ*, *aadB*, *aacC1*, *aacC2*, *aacC3*, *aacA4*, *aac6'-IIa*, *aad(6')*, *ermA*, *ermB*, *ermC*, *mecA*, *vanA*, *vanB*, *vanC*, *satA*, *aac(6')-aph(2'')*, *vat*, *vga*, *msrA*, *sul* and *int*.

40. A diagnostic kit, as defined in claim 29, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 116 and 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of vancomycin B gene and of any bacterium and/or fungus, respectively.

41. A diagnostic kit, as defined in claim 30, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 116 and 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of vancomycin B gene and of any bacterium and/or fungus, respectively.

42. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium and/or fungus, comprising any suitable combination of primers of at least 12 nucleotides in length selected upon alignment of conserved nucleotides of at least two sequences the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof and further comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.

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